

**REMARKS**

In regard to the above amendments to the specification, SEQ ID NO designations are added to the specification based upon matching the sequences in the specification with the corresponding sequences in the Sequence Listing filed concurrently herewith. No new matter is added.

Applicants believe that the present application is now in condition for allowance. Favorable consideration of the application as amended is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

Respectfully submitted,

*August 9, 2002*  
Date

*Jayme A. Huleatt*  
Jayme A. Huleatt  
Reg. No. 34,485

FOLEY & LARDNER  
Customer Number: 22428



22428

PATENT TRADEMARK OFFICE

Telephone: (202) 672-5300  
Facsimile: (202) 672-5399

**THE COMMISSIONER IS HEREBY AUTHORIZED TO CHARGE ANY DEFICIENCY OR  
CREDIT ANY OVERPAYMENT TO DEPOSIT ACCOUNT NO. 19-0741.**

**MARKED-UP SPECIFICATION:**

*Page 9, 1<sup>st</sup> full paragraph:*

In a particularly preferred embodiment of the present invention said polypeptide linker in the polypeptide of the invention comprises 5 amino acids. As demonstrated in the appended examples, said polypeptide linker advantageously comprises the amino acid sequence Gly Gly Gly Gly Ser (SEQ ID NO:11).

*Page 13, 3<sup>rd</sup> full paragraph:*

In addition, said polypeptide sequence capable of selective binding to a solid support can be a positively or a negatively charged amino acid sequence, a cysteine-containing amino acid sequence, avidin, streptavidin, a functional fragment of *Staphylococcus* protein A, GST, a His-tag, a FLAG-tag or Lex A. As is described in the appended Examples, the polypeptide of the invention exemplified by a single-chain antibody has also been expressed with an N-terminal FLAG-tag and/or C-terminal His-tag that allow for easy purification and detection. The FLAG-tag used in the example comprises 8 amino acids (see Figure 8) and is thus preferably used in accordance with the present invention. However, FLAG-tags comprised of shortened versions of the FLAG used in the appended examples such as the amino acid sequence Asp-Tyr-Lys-Asp (SEQ ID NO:12) are suitable as well.

*Page 29, 2<sup>nd</sup> full paragraph:*

Figure 8:  
DNA- and protein-sequence (SEQ ID NOS:9-10) of the bscCD19xCD3 antibody (FLAG-tag containing variant). Numbers indicate the nucleotide (nt) positions, the corresponding amino acid sequence is depicted below the nucleotide sequence. The encoding DNA sequence for the bispecific antibody starts at position 1 and ends at position 1593. The first six nt (position -10 to -5) and the last six nt (position 1596 to 1601) contain the restriction enzyme cleavage sites for EcoRI and SalI, respectively. Nucleotides 1 to 57 specify the leader sequence; nucleotide 82 to 414 and 460 to 831 encode VLCD19 and VHCD19, respectively; nucleotide 847 to 1203 and 1258 to 1575 encode VHCD3 and VLCD3, respectively; and nucleotides 1576 to 1593 encode a His-tag.

*Page 33, the last paragraph which ends on page 34:*

To obtain an anti-CD19 scFv-fragment, the corresponding VL- and VH-regions cloned into separate plasmid vectors served as templates for a VL- and VH-specific PCR using the oligonucleotide primer pairs 5'VLB5RRV/3'VLGS15 and 5'VHGS15/3'VHBspEI, respectively. Thereby, overlapping complementary sequences were introduced into the PCR-products, that combine to form the coding sequence of 15-amino acid (Gly4Ser1)3-linker (SEQ ID NO:13) during the subsequent fusion-PCR. This amplification step was performed with the primer pair 5'VLB5RRV/3'VHBspEI and the resulting fusion product (or rather anti-CD19 scFv-fragment) was cleaved with the restriction enzymes EcoRV and BspEI and thus cloned into the bluescript KS-vector (Stratagene) containing either the (EcoRI/SalI-cloned) coding sequence of the anti-17-1A/anti-CD3 bispecific single-chain antibody with an N-terminal FLAG-tag [1] or that of the modified version without FLAG/epitope (21), thereby replacing the anti-17-1A- by the anti-CD19-specificity and preserving the 5-amino acid (Gly4Ser1)1-linker (SEQ ID NO:11) connecting the C-terminal anti-CD3 scFv-fragment, respectively. Subsequently, the DNA fragments encoding both versions of the anti-CD19/anti-CD3 bispecific single-chain antibody with the domain arrangement VLCD19-VHCD19-VHCD3-VLCD3 were subcloned EcoRI/SalI into the described expression vector pEF-DHRF [1], respectively. The resulting plasmid DNAs were transfected into DHFR-deficient CHO-cells by electroporation: selection, gene amplification and protein production were preformed as described [1]. In the following examples, results obtained with the FLAG-containing version of bscCD19xCD3 are illustrated.